Indian Journal of Natural Products and Resources
Vol. 14(4), December 2023, pp 542-555
DOI: 10.56042/ijnpr.v14i4.6821

# Computational analysis of *Linum usitatissimum*-derived peptides: Toward therapeutic applications

Swetha Sunkar*, Archana V S, Naveen Kumar H and Hariharan V

Department of Bioinformatics, Sathyabama Institute of Science and Technology, Chennai 600119, Tamil Nadu, India

*Received 16 January 2022; revised received 20 November 2023; accepted 22 November 2023*

The food proteome contains diverse bioactive peptides, each offering distinct health advantages for the cardiovascular, nervous, immune, and gastrointestinal systems. This study researches the rich repository of bioactive peptides in flax seeds (*Linum usitatissimum*), focusing on their potential health benefits across diverse physiological systems. Leveraging *in silico* techniques, a comprehensive analysis of 11 protein sequences sourced from UniProt was conducted, utilising enzymatic digestion to generate 160 non-repetitive, non-toxic peptides employing pepsin, trypsin, chymotrypsin, and combined enzymatic strategies via BIOPEP database. The evaluated peptides were scrutinised for a spectrum of biological properties encompassing antimicrobial, antitubercular, and anticancer attributes, alongside an investigation into their immunogenic potential. Docking studies further elucidated the interactions between these peptides and their respective targets, shedding light on their mechanisms of action. The findings unveiled a subset of peptides exhibiting substantial potential across multiple properties, establishing flax seeds as a compelling source of bioactive peptides. This *in silico* predictive approach serves as a vital precursor, offering cues for designing targeted experimental methodologies aimed at extracting therapeutic peptides. The implications of this research extend to the development of functional foods or pharmaceuticals harnessing the bioactive potential of flax seed-derived peptides for promoting human health and well-being.

**Keywords:** Anticancer, Antimicrobial, Bioactive peptides, BIOPEP database, Flax seed, *In silico*

**IPC code; Int. cl. (2021.01)**− A61K 36/00, A61K 131/00, A61P 9/00, A61 31/00, A61K 35/00

## Introduction

In the realm of nutritional science and health research, the spotlight has increasingly turned towards exploring bioactive peptides, miniature marvels embedded within proteins that harbour profound implications for human well-being. These peptides, often overlooked in the intricacies of biological molecules, are emerging as crucial players in various physiological processes, offering a plethora of potential applications in the fields of medicine, nutrition, and biotechnology.

Bioactive peptides refer to specific fragments of amino acid sequences, normally 3-20 amino acids joined by covalent bonds or peptide bonds with low molecular weight. They are found to show a positive influence on precise body functions and may induce preferred health benefits. They are enzymatically released from larger protein precursors during digestion or fermentation. Unlike their parent proteins, these peptides exhibit bioactivity, meaning they can interact with and modulate specific physiological functions within the human body.

Research shows that they were proven to be efficient in regulating disease and promoting health like improvising immunity, lowering blood pressure, lipid and cholesterol-lowering, anti-inflammatory, etc., and hence classified based on their mode of action as antihypertensive, immunomodulatory, antimicrobial, antithrombotic, opioid and antioxidative[1,2]. With the realisation that chemical drugs are likely to induce adverse side effects, bioactive peptides of natural origin are therefore recognised as a plausible alternative, and hence, research is focused on isolating and identifying bioactive peptides. The peptides are in an inactive phase in the parent protein, but when cleaved by enzymes, they tend to display appreciable bioactivity[3]. Moreover, peptides display high stability, efficacy, high selectivity, bioavailable, safe and tolerable, and easy to synthesise and optimise and hence are considered highly promising options for chemically synthesised drugs. These vital properties have gained attention to the extent of developing peptide-based therapeutics in recent years.

---

*Correspondent author
Email: swethauk78@gmail.com
Supplementary table and figures are available online only.

For ages, food-based proteins have been most sought after for being nutritious and functionally important. Still, the recent past has witnessed an enormous research inclination for food-derived peptides and the identification of their bioactivities. Recent studies have identified bioactive peptides in various food sources, highlighting their potential as functional ingredients in developing novel dietary interventions and nutraceuticals. Conventionally, the peptides are produced by fragmenting the source protein using enzymatic hydrolysis to procure peptides, followed by the separation of bioactive peptides by chromatographic methods or membrane separation. This is followed by validating the bioactivity of peptides experimentally, which is a time-consuming and costly process. The bioavailability of these peptides, a critical aspect of their therapeutic efficacy, is also a subject of increasing investigation. Interest in this food-derived peptide developed from the knowledge that natural components from foods offer solutions to disease prevention and management and overall health enhancement[4], especially certain non-communicable diseases like obesity, hypertension and type 2 diabetes[5,6]. This makes them a potential functional diet for promoting health for chronic diseases.

Peptide drugs represent a unique class of pharmaceutical compounds positioned between small molecules and proteins. Within this category, antimicrobial peptides are gaining prominence, characterised by their composition of 10 to 50 amino acids, although, in some instances, they can extend to 100 or more amino acids. Technological advancements have significantly propelled the development of peptide drugs. The discovery of DNA structure, quantitative structure-activity relationship models, recombinant DNA technology, and sequencing technologies have been pivotal[7]. Recombinant technology, exemplified by the approval of insulin synthesis in 1982, has been a major breakthrough, making insulin the top-selling peptide drug[8]. Omics techniques have further contributed to identifying, screening, and characterising peptides from various sources.

Peptide drugs gained popularity due to their competitive advantages, including high selectivity, potency, low toxicity, accumulation in tissues, and broad target range. However, limitations, such as poor oral bioavailability, have shaded their medical applications compared to small molecule drugs and biologics. Strategies like amino acid substitutions, terminus modification, and disulfide bond addition aim to improve pharmacokinetics and pharmacodynamics[9] and can further make peptide-based therapy possible. Understanding the mechanisms of peptide drugs is vital for their development. While antimicrobial peptides have well-reviewed mechanisms, others lack clarity, hindering progress[10]. Clear and novel mechanisms are essential for the future development of peptide drugs. However, the difficulty in the standard procedures in identifying and isolating them offers new avenues for the use of computational approaches that have taken the lead to initially cleave the protein, obtain peptides and then identify the bioactive peptides using various programs.

Computational studies play a central role in advancing peptide generation and analysis by enriching our comprehension of peptide structures and functions. In peptide studies, computational tools offer the capability for de novo design, molecular modelling, virtual screening, and quantitative structure-activity relationship that facilitates the identification of peptide folding, dynamics, stability and functional annotation for novel peptide sequences with desired properties customised for specific functions. Interaction studies, utilising techniques like docking simulations and molecular dynamics, unravel the complexities of peptide-protein interactions, providing predictive details that streamline experimental validation. The significance of computational studies in peptide research expedites the discovery process, offering a cost-effective and time-efficient means to explore the complexities in peptide biology and design[11].

Molecular dynamics simulations, initiated with bovine pancreatic trypsin inhibitor in 1977, have aided in studying challenging membrane-active AMPs[12]. Computational advancements, including machine learning models like QSAR, have emerged as crucial tools in accurately predicting and designing peptide drugs[13,14]. Recent attempts in this area highlight the potential of ML in AMP development[15,16]. These advancements underscore the interdisciplinary nature of peptide drug development, incorporating molecular biology, computational tools, and innovative strategies to address challenges and unlock the full therapeutic potential of peptides.

Research on peptides from foods like milk[17], meat protein-derived peptides[18], tomato seeds[19] giant grouper roe[20], salmon, ricebran[21] and soybean[22] using *in silico* approaches prompted us to explore, derive and identify peptides from flax seeds using *in silico* approach and comprehend their bioactivities and provide cues to possible experimental studies.

## Materials and Methods

### Source protein and enzymes used for *in silico* digestion

Flax seeds *(Linum usitatissimum*/ *Linum humile*) are used as a protein source owing to their nutritional benefits offered to human lives. Eleven reviewed sequences from Flax seeds were retrieved from UniProt and were digested *in silico* using the enzymes pepsin, trypsin, and chymotrypsin.

### *In silico* peptide generation

The peptides were obtained using BIOPEP-UVM, the most widely used program for the digestion and identification of bioactive peptides. The database helps in locating peptides in given protein sequences, simulating proteolysis, and determining the bioactivity of peptides generated and the corresponding parameters. Peptides of different lengths were obtained from this process for the source protein sequences and were used for further analysis.

### PeptideRanker

The peptides obtained from *in silico* hydrolysis were analysed for their potential to exhibit bioactivity using the PeptideRanker tool, which calculates the score for each peptide to assess its activity. The score is calculated based on the neural network and ranges from 0 to 1, where 0 shows the least probability for the peptide to be active while 1 shows the maximum probability.

### Toxicity prediction

The generated peptides were predicted for toxicity using the tool ToxinPred, which uses a dataset containing 1805 toxic peptides. Options are provided to predict the physicochemical properties of peptides.

### Bioactivity prediction

#### *Prediction of anticancer peptides and tumour-homing peptides*

AntiCP and its updated version, AntiCP 2.0, are used to predict the anticancer activity of the generated peptides based on the SVM approach. While the former is based on a dataset containing 225 antimicrobial peptides with anticancer properties, the latter consists of experimentally validated 861 anticancer peptides and 861 non-anticancer or validated antimicrobial peptides, making a much more accurate prediction. TumorHPD is a user-friendly web server that predicts the peptide's tumour homing capacity that is bound to tumour cells as this would enable such peptides to be used for delivery of drugs more specifically to the target and also as imaging agents in diagnostics, thereby making them useful in cancer treatment and management. The prediction is based on the Support Vector Machines model algorithm, which uses a data set with 651 peptides (negative data set) from the SwissProt database and 469 peptides in the main data set.

### Anti-angiogenic property prediction

The peptides are assessed for their ability to act as angiogenesis inhibitors using AntiAngioPred tool that uses the whole amino acid composition-based method of the peptide for the evaluation process.

### Prediction of antimicrobial peptides

Class AMP is the tool used to predict antimicrobial peptides (AMPs). Two algorithms are provided in the program, namely Random Forests (RF) and Support Vector Machines (SVM). The SVM model was selected to predict the antimicrobial properties of the peptides in terms of probability score, which ranges from 0 to 1, indicating the strength of prediction. The higher the probability, the greater the possibility of the prediction being correct. It is available at http://www.bicnirrh.res.in/classamp/. Additionally, the AMP prediction tool from CAMPr3 was used to predict the antimicrobial probability, which is based on 4 algorithms. Peptides, which showed activity by three algorithms, were considered.

### Prediction of antibiofilm activity of peptides

dPABBs web server was used for predicting the antibiofilm activity of peptides based on a main dataset that includes 90 biofilm-active peptides (BAPs) from the BaAMPs database as the positive dataset and 88 quorum sensing peptides (QSPs) from the QSPpred server as the negative dataset. These datasets were used for the calculation of amino acid composition, dipeptide, and tripeptide percentage composition. For the positive independent dataset, 10 peptides were randomly selected from BAPs. The negative independent dataset contains 10 peptides manually curated from published literature. Consequently, 80 BAPs and 88 QSPs have been used for training and testing the Support Vector Machine (SVM) and WEKA-based models.

### Prediction of antitubercular peptides

The ability of the peptides to act as antitubercular peptides has been predicted using four tools, namely iAntiTB, Atbpred and AntiTBPred to better understand the potential of these peptides as antitubercular entities.

### Prediction of Ig-class specific B cell epitopes

Antigenic Peptides with different types of B-cell epitopes that can induce different classes of Antibodies like IgG, IgE and IgA are predicted by the IgPred web server among the submitted peptide queries. This is based on an experimentally validated non-redundant dataset and uses a machine learning approach for prediction, especially for variable length datasets. The accuracy of this model is around 80%.

### Prediction of IFN-gamma-inducing peptides

IFNepitope is a web server to predict and design the epitope, which could induce the release of interferon-gamma. The web server has been developed based on a dataset, which comprises IFN-gamma-inducing and non-inducing MHC class II binders.

### Prediction of IL4-inducing peptides

The SVM-based approach is used for the prediction of peptides that can include IL4 based on amino acid composition, dipeptide composition, amino acid propensity and physicochemical properties. A five-fold cross-validation technique has been used, and evaluation of the performance of different SVM modules has been done by calculating accuracy and Matthew's correlation coefficient.

### Prediction of antihypertensive peptides

AHTp is an *in-silico* method developed to predict and design efficient antihypertensive peptides and is based on an SVM-based machine-learning approach where amino acid descriptors and physicochemical properties are used as parameters for prediction.

### Interaction studies

Some of the peptides which showed bioactive potential to a greater extent with high scores and required length were docked with approved drug targets for certain activities using CLUSPRO and visualised using Pymol. This was carried out as a basic step in understanding the promise offered by these peptides as probable inhibitors for various conditions.

## Result and Discussion

### Source proteins and enzymes used

Flaxseed (*Linum usitatissimum*) has been a part of human lives for a long time. Though initially used for producing fibre, currently, every part of the plant is utilised for several commercial purposes. Research is focused on consuming flax owing to its nutritional properties and potential health benefits, as it houses a lot of biologically active compounds. They are an abundant source of omega-3 fatty acids, α-linolenic acid, short-chain polyunsaturated fatty acids, soluble and insoluble fibres, phytoestrogen lignans, proteins and an array of antioxidants due to the presence of high levels of cysteine and methionine. The protein content of flax is very much comparable to soya proteins and is rich in arginine, glutamic acid and aspartic acid[23]. Reports suggest the ability of flaxseed proteins and their peptides to possess physiological activities like angiotensin-converting enzyme inhibition, anti-diabetic effect, antibacterial activity, and antioxidant capacity. In view of the benefits offered by flaxseed, an attempt has been made to computationally derive peptides and identify their bioactivities *in silico*, as this would provide a cue to experimental validation. It may further document some probable therapeutic peptides. Considering this perspective, key enzymes—pepsin, trypsin, and chymotrypsin—were employed to simulate gastrointestinal conditions through *in silico* protein hydrolysis. Eleven reviewed sequences of flax seeds were obtained from Uniprot and used for deriving peptides. The uniport IDs of these proteins are Q4R0H9, Q40253, E6Y2X0, P93243, P82381, P48417, Q4R0I0, P32062, Q40255, P0DKC8, P81785, P69315.

### *In silico* peptide generation and analysis of bioactive potential

Biopep (BIOPEP-UWM Database of Bioactive and Sensory Peptides) has been used to digest the flaxseed proteins into smaller peptides of different sizes[24]. The proteolytic enzymes pepsin (pH 1.3), trypsin and chymotrypsin were used individually and in combination for this process as these enzymes are expected to produce peptides with good bioactivity[19]. A total of 1290 peptides were generated, from which duplicates were removed and analysed by Peptide Ranker to obtain the peptides with potential activity. A minimum threshold of probability 0.5 is set as selection criteria, as a higher threshold may lead to the omission of positives. About 160 peptides were predicted to show activity, of which 25 were dipeptides, 47 were tripeptides, and the remaining 88 peptides were of the size 4 and above amino acids.

### Toxicity prediction

Even though peptides have several advantages over small molecules in therapeutics, such as good bioactivity, low production cost, high specificity, and high penetration[25,26], their toxicity and stability continue to be a concern. Therefore, assessing the toxicity of the peptides could be considered a critical step in bioactive peptide identification and therapy. Hence, the generated 160 peptides were analysed using Toxinpred. The tool predicted that all the 160 peptides were found to be non-toxic based on the SVM score and, therefore, predicted for bioactivities. The general properties of the peptides were also predicted, like molecular weight, charge, pI, hydrophobicity, hydrophilicity, steric hindrance and side bulk properties and are provided as supplementary Table 1. The preliminary analysis showed that the molecular weight of 15 peptides was in the range 1000 to 2900, 34 peptides in the range 700-999, 62 peptides in the range 500-699 and 49 peptides in the range 260-499. Molecular weight is a key factor in the development of drugs as they influence permeability and absorption[27].

Likewise, 33 peptides were found to be positively charged, 39 peptides were found to be negatively charged, and 88 peptides were found to be neutral. Charge and molecular weight are two important factors that affect the permeability of the peptide. Hydrophilicity is another important parameter for peptides as the paracellular pathway is identified as an aqueous route, influencing bioavailability. Gastrointestinal digestive stability is influenced by the changes in hydrophobicity; hence, determining these factors is mandatory to understand the basic properties of peptides[28]. The results are provided in Supplementary Table 1.

### Bioactivity prediction

#### *Prediction of antimicrobial peptides*

The ability of peptides to act as antimicrobial agents was analysed using the ClassAMP tool[29]. This tool uses the SVM approach and predicts antibacterial, antiviral, and antifungal activities of peptides along with score. Of late, SVM-based methods, a classification system based on the supervised learning approach, have been proven to produce better results with low prediction errors compared to that of artificial neural networks. They also work well with large datasets with many dimensions with small samples and are good at building classifiers[30]. In this study, scores greater than 0.75 were considered to identify peptides with better activity potential. All 160 peptides were found to be antimicrobial, where 111 peptides showed an antibacterial nature, 36 peptides showed antifungal properties, and 12 peptides showed antiviral properties. iAMPpred was another tool used to make predictions based on machine learning and physicochemical and structural features and gives the probability from 0 to 1 where 0.7 was used as the minimum value[31], and the results predicted 24, 4 and 16 peptides to possess antibacterial, antifungal and antiviral properties. These results also indicated that certain peptides were showing potential for all three activities.

A tool exclusively for the prediction of antiviral peptides, namely Meta-iAVP based on feature representation, was used for further analysis, which predicted 33 peptides to be antiviral whose value was above 0.7[32]. A comparative analysis of these results has pointed out some common peptides, namely AISIPWGVK, VCGL, HYCGL, HGL, WCL, VWSCHR, and PIF, that had good probability values in all three tools and can be considered as potential antiviral agents.

The antifungal potential was displayed by a total of 42 peptides based on the results obtained from

Table 1 — Antimicrobial peptides obtained *in silico* from Flax seed proteins

| Property | List of Peptides |
|---|---|
| Antibacterial and Antiviral Peptides | VWSCHR, DWK, HGL, WCL, PIF, SDCWR, DCPR, GWHIGK, ISGGTF, AGHML, MGAL, PGL, CQPGF, AGYF, GGL, DYF, AVCF, WIGR, YL, CPGK, GPF, WVL, VCGL, TPWAIWTR, CR, GL, VLGGMQIF, GGYF, AIML, GF, GPL, MIYL, WL, CPK, YHIYYEGCL, IF |
| Antifungal and Antibacterial | MGR, CPGK, AVCF, MAAGF, MYR, HGL, GWHIGK, DIGGCPDL |
| Antifungal and Antiviral | MAAGF, GL, DF, IPSEF, AVCF, CPGK, HGL, WPDQQQL, GPDVNEADYVAWVCDQDAYACSGQK, GWHIGK |
| Antifungal, Antiviral and Antibacterial | GL, CPGK, AVCF, MAAGF, HGL, GWHIGK |

ClassAMP, iAMPPred and AntiFp tools. AntiFp tool, whose accuracy is up to 88%[33] was used to predict solely the antifungal peptides based on the SVM method, and the results showed that two peptides, namely MGR and MYR, were found to have high scores (1.2 and 1.08, respectively) for antifungal activity. One peptide, HGL, was found to show high values for antifungal activity in the tools used. A total of 41 peptides were found to possess antifungal properties based on the tools used for prediction.

Using ClassAMP and iAMPpred, 111 and 24 peptides were found to possess antibacterial properties, and interestingly, 19 peptides were found to be common in both, thereby making them promising in the field of antibacterial research. This result convinces the fact that flax, which is a nutritious food, can be considered to house many peptides having antimicrobial properties. There were 36 peptides that showed both antibacterial and antiviral properties, 8 peptides that showed antifungal and antibacterial properties and 10 peptides that showed antifungal and antiviral potential. Six peptides were found to show all three properties, making them very important in the quest for bioactive peptides, and the results are provided in Table 1.

Three peptides, namely HGL, AVCF, and CPGK, were identified as having the inherent potential to exhibit a diverse array of antimicrobial activities and be potential candidates for experimental assessments.

### Prediction of antibiofilm activity of peptides

Biofilm, as a growth adaptation mechanism to environmental stress, enables the organisms to resist stressors, making them difficult to treat and eradicate[34]. It was reported by the National Institutes of Health that 75% of infectious diseases in humans are caused by bacterial biofilms, which include wound infections, lung infections, urinary tract infections, etc. Bacteria embedded in the biofilm matrix are known to decelerate metabolism, confer antibiotic resistance and are found to be 1000 times resistant to antibiotics and in certain cases, lead to persistent infection with poor prognosis, increasing health expenses[35]. This necessitates an urgent need to find alternative approaches to treat and prevent biofilm infections. One favourable alternative is the antibiofilm peptides, which are based on a new property of cationic, amphipathic peptides[36]. The disruption of biofilms is a constant area of research, and in this line, the ability of peptides to inhibit the biofilm formation is assessed using the tool dPABBs, which uses two types of algorithms, namely SVM and Random Forest algorithm (WEKA), taking into consideration the whole amino acid composition[37]. The 160 peptides obtained were submitted to dPABBs using both algorithms. While SVM is a supervised machine learning approach, Random Forest is a classification algorithm which is non-parametric, efficient, achieving high accuracy and poses the advantage of handling small sample sizes, complex data structures and many-dimensional feature space.

Based on the results, a total of 26 peptides were found to have the potential for biofilm inhibition. The minimum value for the SVM score was considered to be 0.7 while the WEKA probability was 1, based on which it has been observed that 9 peptides, namely WR, WK, WIGR, CR, PWK, GWHIGK, MYR, PR, PPR were found to possess antibiofilm activity. Their SVM score ranged from 0.7 to 1. These peptides were also found to possess significant antibacterial potential, making them promising candidates for further studies. Interestingly, MYR is also found to possess good antifungal potential. These results provide a cue to various experimental studies on using peptides as antibiofilm agents. Darwish *et al.*[38] initially identified antibiofilm peptides using an *in silico* hybrid approach, which was later followed by experimental validation.

As a preliminary study, docking has been performed with the peptide GWHIGK with that of acyl-homoserine lactone synthase LasI (AHL), which is the most common target for antibiofilm drugs for *Pseudomonas aeruginosa* infections to comprehend the ability of the peptide to interact with AHL. CLUSPRO was used to carry out the protein-peptide docking, and the results are visualised using Pymol. The interaction energy was found to be the lowest (-583.8 kcal/mol) for this peptide-protein complex of all the models generated with 5 polar contacts, indicating the possibility of being stable. The figure illustrating the interactions is provided in Supplementary Fig. 1.

### Antitubercular peptides

The resistance to existing antibiotics by human pathogens, specifically *Mycobacterium tuberculosis* causing infectious tuberculosis, is a matter of concern, and there is always a quest to identify novel compounds that can cater to this problem. In this context, growing interest is seen in peptides as they can show specific affinity toward cell envelope and less immunogenicity and diverse modes of action[39]. Therefore, this study tried to analyse the peptides obtained from flax to show any antitubercular activity. Tools, namely iAntiTB, AtbPpred, and AntiTbPred, were used for prediction, and the results are provided in Table 2. AntiTbPred predicted 30 peptides to show antitubercular potential, of which 14 displayed an SVM score greater than 0.7. iAntiTB gives the probability score based on SVM and random forest algorithms incorporating sequence-based features and predicts high, medium and low confidence anti tuberculosis peptides[40]. A total of 71 peptides showed high and medium confidence, of which 51 showed high confidence. Based on the minimum score of 0.1, 13 sequences were found to possess high confidence (Table 2). AtbPpred tool predicted that 9 sequences were found to display antitubercular potential whose score was in the range of 0.5 to 0.73. Three sequences were found to have a score greater than 0.7. Certain peptides were found to be common in all three tools, which are YHIYYEGCL, HAPTGSWEIF, NWGTNL, TPWAIWTR, AISIPWGVK, IYQCTEPTWL, ITWPDQPYR, QIPGDYGL and can be considered as potential candidates for experimental validation.

Table 2 — Antitubercular peptides obtained *in silico* from Flax seed proteins

| Tool | Peptide sequence | SVM Score | Prediction |
|---|---|---|---|
| AntiTbPred | IWSVYEF | 1.7590191 | Antitubercular peptide |
| | AGHML | 1.3940922 | Antitubercular peptide |
| | ISMPAHL | 1.3710355 | Antitubercular peptide |
| | YHIYYEGCL | 1.2878429 | Antitubercular peptide |
| | IYQCTEPTWL | 1.2546404 | Antitubercular peptide |
| | HYCGL | 1.2484649 | Antitubercular peptide |
| | APIL | 1.2254642 | Antitubercular peptide |
| | HAPTGSWEIF | 1.1486746 | Antitubercular peptide |
| | NWGTNL | 1.086506 | Antitubercular peptide |
| | DYVIPEF | 1.0426231 | Antitubercular peptide |
| | NAWPEL | 0.82559656 | Antitubercular peptide |
| | WR | 0.79438758 | Antitubercular peptide |
| | AIML | 0.75180465 | Antitubercular peptide |
| | MGAL | 0.7456657 | Antitubercular peptide |
| | TPWAIWTR | 0.71429 | AtbP |
| | AISIPWGVK | 0.73214 | AtbP |
| | GAYASDPF | 0.70357 | AtbP |
| | ADMMR | 0.59643 | AtbP |
| | HAPTGSWEIF | 0.54286 | AtbP |
| | IYQCTEPTWL | 0.53929 | AtbP |
| | DMPPASPSEDESSVWWK | 0.53571 | AtbP |
| | ITWPDQPYR | 0.52143 | AtbP |
| | QIPGDYGL | 0.5 | AtbP |
| iAntiTb | Peptide Sequence | Probability Score | Prediction |
| | YHIYYEGCL | 0.321 | High Confidence AntiTB |
| | AISIPWGVK | 0.219 | High Confidence AntiTB |
| | ITWPDQPYR | 0.218 | High Confidence AntiTB |
| | TPWAIWTR | 0.185 | High Confidence AntiTB |
| | VWSCHR | 0.141 | High Confidence AntiTB |
| | NWGTNL | 0.137 | High Confidence AntiTB |
| | IYQCTEPTWL | 0.134 | High Confidence AntiTB |
| | EHSHITADDWGGWR | 0.132 | High Confidence AntiTB |
| | SDCWR | 0.123 | High Confidence AntiTB |
| | WIGR | 0.116 | High Confidence AntiTB |
| | GWHIGK | 0.113 | High Confidence AntiTB |
| | GW | 0.108 | High Confidence AntiTB |
| | QIPGDYGL | 0.106 | High Confidence AntiTB |
| | AW | 0.096 | High Confidence AntiTB |

The interaction between the potential peptides and that of the target was carried out using the CLUSPRO tool and visualised using Pymol. The most common target for tuberculosis drugs is Enoyl-(acyl-carrier-protein) reductase (ENR), which is a limiting step enzyme in the Fatty Acid Synthase II system of *Mycobacterium tuberculosis*. This protein structure has been obtained from a Protein data bank (PDB) with the ID 1BVR and used for docking with potential antitubercular peptides, and the results are provided in Table 3. The peptides showed good interaction with the least possible energies with polar contacts, indicating the promise offered by these peptides to act as candidate antitubercular peptides (Supplementary Fig. 1).

The peptide IYQCTEPTWL emerged as a consistently and significantly predicted candidate across all computational tools. Notably, its interaction profile revealed a noteworthy binding affinity characterised by polar contacts and a correspondingly low binding energy. This compelling observation strongly advocates for the potential utility of this peptide in subsequent investigations, positioning it as a promising candidate for further exploration in the context of tuberculosis management.

### Prediction of anticancer peptides and tumour-homing peptides

The 160 peptides were analysed for their probability to home tumours using TumorHPD, and it was observed that 112 peptides were considered as tumour homing peptides based on the SVM score, whose cut-off was considered beyond 0.75. A few of the peptides showed a much higher value of 2.65, indicating the potential of these peptides to house tumours. Peptides having anticancer potential were predicted using two tools, namely AntiCP and its version AntiCP 2.0, developed by IIIT Delhi[41]. The former is based on the SVM approach and was able to take up even di and tri peptides, while the latter followed a machine learning algorithm which made a prediction for peptides longer than three peptides. The minimum score for prediction was given as 0.7 in both cases. The results obtained showed that of 160 peptides, 97 peptides (AntiCP) and 12 peptides (AntiCP 2.0) were found to show potential for anticancer activity with MGAL, TGDF, PNTF, PPPHGSIL, ISMPAHL, YHIYYEGCL, MGDAL, DIACF peptides common for both the tools. This study suggests that flax seeds possess a significant number of peptides that can have anticancer activity. A similar approach has been adopted by Fatemi *et al.*[42] to initially identify the anticancer peptides from Human Lactoferrin Protein, followed by experimental studies to validate the potential of the peptides.

Table 3 — Potential antitubercular peptides and their interaction with IBVR

| Peptide | Lowest energy | No of polar contacts |
|---|---|---|
| AISIPWGVK | -972.9 | 5 |
| HAPTGSWEIF | -953.1 | 3 |
| ITWPDQPYR | -825.8 | 9 |
| IYQCTEPTWL | -1094.1 | 7 |
| NWGTNL | -710.2 | 9 |
| QIPGDYGL | -733.1 | 5 |
| TPWAIWTR | -957.6 | 5 |
| YHIYYEGCL | -1025.7 | 7 |

Studies revealed that anticancer potential peptides contained leucine, lysine and glycine as they can travel through the membrane through snorkeling thereby disrupting cell membrane integrity and leading to cancer cell toxicity[43,44]. Also, the peptides were evaluated for their tumour-homing capacity using TumorHPD, where 128 peptides were found to show the potential, of which 84 peptides had a score of more than 0.7. About 27 peptides showed a score in the range of 0.7 to 1, 50 peptides in the range of 1 to 2 and 7 peptides had a score greater than 2 indicating the promise that these peptides exhibit in inhabiting tumours. It was reported that glutamic acid and aspartic acids in the peptides presented potential anti-proliferative activity in the tumour cells[45]. This study has also identified certain peptides from flax seeds with the above amino acids, thereby making them probable candidates for further analysis. It is interesting to note that some peptides displayed the ability to home tumours and induce anticancer activity (Table 4).

Table 4 — Common anticancer peptides and tumour homing peptides obtained *in silico* from Flax seed proteins

| Peptide sequence | SVM score | Peptide sequence | SVM score | Peptide sequence | SVM score |
|---|---|---|---|---|---|
| TPWAIWTR | 1.27 | PPPHGSIL | 0.92 | WK | 0.8 |
| YEF | 1.23 | CA | 0.92 | PGL | 0.79 |
| TCF | 1.12 | PDDYF | 0.89 | SMR | 0.79 |
| DCCCF | 1.11 | VWSCHR | 0.89 | SPR | 0.78 |
| SECF | 1.11 | PPK | 0.86 | PSL | 0.74 |
| IHF | 1.1 | ISMPAHL | 0.85 | PVSF | 0.73 |
| CPGK | 1.1 | SVPF | 0.84 | PPR | 0.73 |
| SSSF | 1.08 | YGR | 0.84 | DCPR | 0.73 |
| PR | 1.03 | IPL | 0.83 | PGM | 0.73 |
| ADMMR | 1.01 | CDR | 0.83 | AVCF | 0.72 |
| IYQCTEPTWL | 1 | ITWPDQPYR | 0.83 | AMMK | 0.72 |
| WPF | 0.99 | MGR | 0.82 | PIF | 0.72 |
| WR | 0.99 | CQPGF | 0.82 | HAF | 0.72 |
| DYF | 0.98 | MMADTCL | 0.81 | DMGR | 0.72 |
| CPK | 0.95 | CL | 0.8 | PSGSF | 0.72 |

### Anti-angiogenic property prediction

The quest for novel molecules for the treatment of cancer is incessant. One major process in the development of cancer is angiogenesis, which, when curtailed, would lead to loss of blood supply to the growing cells that eventually results in inhibition of cell proliferation. The peptides generated were evaluated for their ability to act as angiogenesis inhibitors using the tool AntiAngioPred, which is based on the SVM approach[46]. Of the 160 peptides generated, it was observed that 39 peptides showed antiangiogenesis properties, but only 18 peptides showed SVM scores above 0.7, in which 12 peptides showed high SVM values that were in the range of 1 to 3.3. Another sequence-based prediction using a random forest algorithm with the inclusion of amino acid composition, physicochemical properties, disulphide composition, pseudo amino acid composition, and amphiphilicity was carried out employing the TargetAntiAngio tool (Table 5). The result predicted that 39 peptides showed anti-angiogenic properties, of which 22 peptides had a score greater than 0.7. It was interesting to observe that certain peptides were common to both the tools, which include DCCCF, CQPGF, MIDDPR, and ISSCF, indicating the high probability that these peptides could serve as cues to *in vitro* studies.

The interaction between the potential anti-angiogenic peptides with that of the target 1VPF (Vascular endothelial growth factor), which is the most common target for angiogenic drugs[47] was performed using CLUSPRO and the results are provided in Table 6 and Supplementary Fig. 1. The results present good interactions with less energies making the complex stable which might possibly bring about an inhibitory effect and hence can be considered for further studies. Five peptides, DCCCF, CQPGF, MIDDRP, ISSCF and PDDYF, were found to be commonly predicted by the tools utilised and additionally were also found to have good interactions with the target with low binding energies. Interestingly, one two peptides, DCCCF and CQPGF, were found to possess tumour-homing and anti-angiogenic potential, which clearly states the possibility of using these peptides for further experimental studies.

Table 5 — Anti-angiogenic peptides obtained *in silico* from Flax seed proteins

| TargetAntiAngio | | AntiAngioPred | | Prediction |
|---|---|---|---|---|
| Peptide Sequence | SVM Score | Peptide Sequence | SVM Score | |
| DCPR | 0.848 | SDCWR | 3.3 | Anti-angiogenic |
| CPGK | 0.838 | DCCCF | 2.3 | Anti-angiogenic |
| CPK | 0.824 | CWQPQDF | 2.04 | Anti-angiogenic |
| SPR | 0.816 | ITWPDQPYR | 1.98 | Anti-angiogenic |
| PPR | 0.784 | PDDYF | 1.82 | Anti-angiogenic |
| CDR | 0.776 | TPWAIWTR | 1.7 | Anti-angiogenic |
| PR | 0.762 | CQPGF | 1.39 | Anti-angiogenic |
| MIDDPR | 0.756 | MIDDPR | 1.37 | Anti-angiogenic |
| CR | 0.752 | HYCGL | 1.19 | Anti-angiogenic |
| GR | 0.742 | VWSCHR | 1.13 | Anti-angiogenic |
| CQPGF | 0.742 | GWHIGK | 1.1 | Anti-angiogenic |
| PPK | 0.736 | WPDQQQL | 1.03 | Anti-angiogenic |
| WR | 0.734 | IPSEF | 0.95 | Anti-angiogenic |
| SMR | 0.734 | DIACF | 0.89 | Anti-angiogenic |
| CA | 0.732 | NSWGGF | 0.88 | Anti-angiogenic |
| PSL | 0.724 | IYQCTEPTWL | 0.79 | Anti-angiogenic |
| ISSCF | 0.722 | ISSCF | 0.72 | Anti-angiogenic |
| PSGSF | 0.716 | YHIYYEGCL | 0.71 | Anti-angiogenic |
| WIGR | 0.714 | | | Anti-angiogenic |
| MGR | 0.712 | | | Anti-angiogenic |
| DCCCF | 0.706 | | | Anti-angiogenic |
| MYR | 0.7 | | | Anti-angiogenic |

Table 6 — Potential anti-angiogenic peptides and their interaction with 1VPF

| Peptide | Lowest energy | No of polar contacts |
|---|---|---|
| CQPGF | -531.1 | 2 |
| **ISSCF** | -531.7 | 9 |
| MIDDRP | -436.5 | 3 |
| PSGSF | -480.2 | 3 |
| SDCWR | -580.0 | 5 |
| DCCCF | -673.1 | 4 |
| CWQPQDF | -651.1 | 5 |
| ITWPDQPYR | -653.9 | 5 |
| PDDYF | -578.4 | 7 |
| TPWAIWTR | -733.2 | 3 |

### Immunogenic peptides prediction

#### *Prediction of Ig-class specific B cell epitope*

The peptides were analysed for their properties to induce antibodies like IgG, IgE and IgA using the web server IgPred[48]. This motif-based and SVM-based approach utilised amino acid composition and propensity physico-chemical properties of amino acids as key factors for analysis. Of the 160 peptides submitted, only three peptides, DF, HGEDDPPL, and GPDVNEADYVAWVCDQDAYACSGQK were found to possess IgG epitope with a score of 1.00, 1.027, and 0.83, respectively, based on the set threshold of 0.7 (Table 7). IgG is the most common type of antibody and is found in blood and extracellular fluid. It is mainly involved in controlling infections caused by viruses, bacteria and fungi. This

Table 7 — Immunogenic peptides obtained *in silico* from Flax seed proteins

| Sequence | Result | Score |
|---|---|---|
| | IgPred | |
| DF | IgG Epitope | 1.000 * |
| HGEDDPPL | IgG Epitope | 1.027 * |
| GPDVNEADYVAWVCDQDAYACSGQK | IgG Epitope | 0.835 * |
| | IL4pred | |
| GPDVNEADYVAWVCDQDAYACSGQK | IL4 inducer | 1.13 |
| | IFNepitope | |
| GPDVNEADYVAWVCDQDAYA CSGQK | POSITIVE | 2.7461466 |
| AGL | POSITIVE | 1.7076737 |
| GW | POSITIVE | 1.6046283 |
| GL | POSITIVE | 1.5999261 |
| PL | POSITIVE | 1.5855054 |
| PR | POSITIVE | 1.5814303 |
| IPL | POSITIVE | 1.5581142 |
| CA | POSITIVE | 1.5531743 |
| WR | POSITIVE | 1.5447632 |
| WK | POSITIVE | 1.5438279 |
| QGL | POSITIVE | 1.5438229 |
| DGL | POSITIVE | 1.5415291 |
| PPR | POSITIVE | 1.5303338 |
| VW | POSITIVE | 1.5275715 |
| CR | POSITIVE | 1.5238086 |
| CL | POSITIVE | 1.5234774 |
| ML | POSITIVE | 1.5164348 |
| WL | POSITIVE | 1.5109999 |
| PF | POSITIVE | 1.5060585 |
| AW | POSITIVE | 1.5057406 |
| TF | POSITIVE | 1.5055962 |
| GF | POSITIVE | 1.5055469 |
| YL | POSITIVE | 1.5052928 |
| AF | POSITIVE | 1.5047028 |
| EF | POSITIVE | 1.5029449 |
| SF | POSITIVE | 1.5009998 |
| DF | POSITIVE | 1.4999066 |
| DWK | POSITIVE | 1.4942247 |
| GGL | POSITIVE | 1.491282 |
| NF | POSITIVE | 1.48933 |
| GR | POSITIVE | 1.4883642 |
| HGL | POSITIVE | 1.4854016 |
| WF | POSITIVE | 1.4853954 |
| IF | POSITIVE | 1.480538 |
| PWK | POSITIVE | 1.4790976 |
| AAF | POSITIVE | 1.4686916 |
| PAR | POSITIVE | 1.4577758 |
| MYR | POSITIVE | 1.4548261 |
| SMR | POSITIVE | 1.4487212 |
| DYF | POSITIVE | 1.4351772 |
| PSGSF | POSITIVE | 1.4317539 |
| CDR | POSITIVE | 1.4120339 |
| CPK | POSITIVE | 1.3844415 |
| YGR | POSITIVE | 1.3801481 |
| TDF | POSITIVE | 1.3785849 |
| PSL | POSITIVE | 1.3767201 |
| SPR | POSITIVE | 1.3729967 |
| WCL | POSITIVE | 1.3681897 |
| GML | POSITIVE | 1.3506476 |
| WVL | POSITIVE | 1.350281 |
| WPF | POSITIVE | 1.3479317 |
| PGL | POSITIVE | 1.3440957 |
| HAF | POSITIVE | 1.3364758 |
| ISF | POSITIVE | 1.3205965 |
| VCGL | POSITIVE | 1.314411 |
| PML | POSITIVE | 1.3122416 |
| MGR | POSITIVE | 1.3025589 |
| DCPR | POSITIVE | 1.3000639 |
| PNL | POSITIVE | 1.293201 |
| TCF | POSITIVE | 1.2930514 |
| MAL | POSITIVE | 1.2837949 |
| SSSF | POSITIVE | 1.2817588 |
| GGM | POSITIVE | 1.2800246 |
| NNF | POSITIVE | 1.2768381 |
| STF | POSITIVE | 1.2572905 |
| AQDF | POSITIVE | 1.2515858 |
| PIF | POSITIVE | 1.2489583 |
| PPL | POSITIVE | 1.2313134 |
| DGF | POSITIVE | 1.2301488 |
| QIF | POSITIVE | 1.2275881 |
| IHF | POSITIVE | 1.2246544 |
| YEF | POSITIVE | 1.2090749 |
| PGM | POSITIVE | 1.207556 |
| TNF | POSITIVE | 1.2071553 |
| GPF | POSITIVE | 1.2025912 |
| GPL | POSITIVE | 1.179452 |
| AMMK | POSITIVE | 1.1598673 |
| ATF | POSITIVE | 1.1388601 |
| PSEF | POSITIVE | 1.1367212 |
| WIGR | POSITIVE | 1.1247194 |
| DCCCF | POSITIVE | 1.0884795 |
| APIL | POSITIVE | 1.0682786 |
| MSYL | POSITIVE | 1.0519766 |
| QNAF | POSITIVE | 1.0417373 |
| SDCWR | POSITIVE | 1.0161349 |
| SVPF | POSITIVE | 1.0146763 |
| SWIF | POSITIVE | 1.0116286 |
| MIYL | POSITIVE | 1.0104446 |
| PMVL | POSITIVE | 1.0102917 |
| DMGR | POSITIVE | 1.0089425 |
| SCAF | POSITIVE | 1.0038919 |
| TGDF | POSITIVE | 0.99931086 |
| AVCF | POSITIVE | 0.98723566 |

(*contd.*)

Table 7 — Immunogenic peptides obtained *in silico* from Flax seed proteins (*contd.*)

| | | |
|---|---|---|
| YDSF | POSITIVE | 0.98535979 |
| HNPF | POSITIVE | 0.97989542 |
| PNTF | POSITIVE | 0.97533882 |
| PPK | POSITIVE | 0.96413102 |
| NGEPF | POSITIVE | 0.9364375 |
| TMYF | POSITIVE | 0.925063 |
| SSQDF | POSITIVE | 0.9201417 |
| MGAL | POSITIVE | 0.91794482 |
| CPGK | POSITIVE | 0.91674058 |
| AIML | POSITIVE | 0.8846983 |
| PDDYF | POSITIVE | 0.8603234 |
| SECF | POSITIVE | 0.84332394 |
| HMMK | POSITIVE | 0.84275153 |
| IPNL | POSITIVE | 0.83497672 |
| GGYF | POSITIVE | 0.82638316 |
| GWHIGK | POSITIVE | 0.81410718 |
| GGDL | POSITIVE | 0.813564 |
| PVSF | POSITIVE | 0.80866207 |
| GSSHF | POSITIVE | 0.79597542 |
| GMDPAR | POSITIVE | 0.77877297 |
| GYQPF | POSITIVE | 0.76720568 |
| IPSEF | POSITIVE | 0.75146022 |
| AGYF | POSITIVE | 0.72400914 |
| AAGF | POSITIVE | 0.70058084 |

preliminary study provides a cue to developing peptide-based immune-therapeutics.

**Prediction of IL4-inducing peptides**

Interleukin 4 (IL4) is a cytokine that has many biological functions, which include the stimulation of activated B-cell and T-cell proliferation and the differentiation of B cells into plasma cells. It is an important regulator of humoral and adaptive immunity. Therefore, identifying sources that can induce the production of IL4 is definitely required. When assessed for IL4-inducing capability using IL4pred[49], of the 160 peptides, only one peptide, GPDVNEADYVAWVCDQDAYACSGQK, showed an SVM score of 1.13 which is again the same peptide that showed potential for inducing INF gamma and IgG (Table 7). This preliminary data analysis reveals the possibility that flax seeds possess certain peptides that can induce the immunogenic response and act. Still, experimental validation must be carried out to develop peptide-based therapeutics.

**Prediction of IFN-gamma-inducing peptides**

The ability of peptides to induce the production of IFN gamma was assessed using the IFNepitope tool, which is based on a hybrid approach involving a motif-based approach (MERCI) and an SVM-based method whose prediction accuracy is 82%[50]. The results indicated that of the 160 peptides, 142 peptides showed a positive result based on the score (Table 7). The cutoff value of 0.7 for the score has been considered to find peptides with better activity. The peptide with the maximum SVM score was found to be GPDVNEADYVAWVCDQDAYACSGQK with a score of 2.7, while 90 peptides showed a score in the range of 1 to 2, and the remaining 16 sequences had a score in the range of 0.7 to 1. IFNγ, or type II interferon, a cytokine, is important for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFNγ is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression. Anomalous IFNγ expression is associated with several autoinflammatory and autoimmune diseases. The importance of IFNγ in the immune system stems in part from its ability to inhibit viral replication directly and, most importantly, from its immuno-stimulatory and immunomodulatory effects.

It is noteworthy that the two peptides that displayed the potential to induce IgG immunoglobulins, namely GPDVNEADYVAWVCDQDAYACSGQK and DF, are also found to show potency to induce INF gamma, and the former peptide showed potential to induce IL4 which suggests that these peptides can be studied further.

**Prediction of antihypertensive peptides**

The peptides generated were assessed for their ability to act as antihypertensive peptides using the tool AHTpin, and the results are provided in Table 8. A total of 160 peptides were checked, and only 88 peptides displayed the potential, of which only 12 peptides had an SVM score greater than 0.7. The property of the peptides is calculated based on datasets obtained from AHTPDB, BIOPEP and ACEpepDB, using which descriptors are defined, which are amino acid composition, atomic composition and chemical descriptors. These are used as parameters to train SVMs, and the performance is evaluated using Pearson's and Mathew's Correlation Coefficient.

The interaction between the potential antihypertensive peptides with that of the target 1O86 (Human Angiotensin Converting Enzyme (ACE) was performed to analyse the nature of bonds formed using CLUSPRO, and the results are provided in Table 9. ACE is the most common target for blocking the renin-angiotensin system by many

Table 8 — Antihypertensive (AHT) peptides obtained *in silico* from Flax seed proteins

| Peptide | SVM | Prediction |
|---|---|---|
| DYVIPEF | 1.45 | AHT |
| HYCGL | 1.28 | AHT |
| PMVL | 1.22 | AHT |
| CQPGF | 1.15 | AHT |
| GYQPF | 1.13 | AHT |
| HGEDDPPL | 1.03 | AHT |
| SVPF | 0.9 | AHT |
| PVSF | 0.9 | AHT |
| APIL | 0.86 | AHT |
| DMPPASPSEDESSVWWK | 0.85 | AHT |
| GAYASDPF | 0.79 | AHT |
| CPGK | 0.75 | AHT |

Table 9 — Potential antihypertensive peptides and their interaction with 1O86

| Peptide | Lowest energy | No of polar contacts |
|---|---|---|
| CQPGF | -552.8 | 4 |
| DMPPASPSEDESSVWWK | -769.5 | 15 |
| DYVIPEF | -683.7 | 8 |
| GAYASDPF | -656.6 | 8 |
| GYQPF | -599.0 | 2 |
| HGEDDPPL | -574.4 | 7 |
| HYCGL | -676.3 | 4 |

antihypertensive drugs as they are involved in the lowering of angiotensin II[51]. The results are provided in Table 9 and Supplementary Fig. 1, which indicate better interaction as these peptide-protein complex showed the least energies with good polar contacts. These results promise the use of these peptides for further experimental studies to make them plausible candidates for therapeutic purposes.

Even though peptides are specific, selective, well tolerated, relatively safe and efficient, all peptides are not suitable for direct therapeutic use owing to their inherent limitations like short plasma half-life, weak physical and chemical stability due to the internal conditions and enzymatic degradations. These issues are now being addressed gradually by either modifying the structures using a rational drug design approach or conjugating with other molecules which can offer stability or protect the peptide from digestion by identifying the cleavage sites and modifying them, all this leading to the development of a better version of the peptide as a plausible drug. This study could, therefore be a first step in identifying possible peptides from the food consumed that is flax seeds and act as cue in developing therapeutic multifunctional peptides.

## Conclusion

In conclusion, the traditional methods for discovering bioactive peptides have historically been labour-intensive and time-consuming, yielding only a limited number of therapeutic peptides from diverse food sources. However, the recent advancements in computational approaches for identifying protein structures and functions have revolutionised the process, offering a more efficient means of identifying bioactive peptides through *in silico* digestion of proteins and assessing their potential activities. This present study successfully extracted and identified promising peptides from flaxseed proteins, showcasing a spectrum of bioactivities. Some peptides exhibited antimicrobial properties, while others demonstrated immunogenicity. Additionally, certain peptides displayed significant anticancer potential, and others showed promise in antihypertensive and antitubercular activities. Remarkably, several peptides, including DMPPASPSEDESSVWWK, DCCCF, CQPGF, MIDDRP, ISSCF, IYQCTEPTWL, CPGK, HGL, AVCF, TPWAIWTR, GWHIGK, IYQCTEPTWL, CQPGF, and PSGSF, exhibited multiple activities. This diverse range of functionalities positions flaxseed as a promising source for developing therapeutic peptides. The *in silico* evaluation presented herein serves as a valuable foundation for further experimental validation, paving the way for developing novel therapeutic peptides. By bridging computational predictions with empirical evidence, this study opens avenues for exploring and utilising flaxseed-derived peptides in developing innovative and effective therapeutic interventions.

## Conflict of interest

The authors have no conflicts of interest.

## References

1 Sánchez A and Vázquez A, Bioactive peptides, A review, *Food Quality Saf*, 2017, **1**, 29–46.

2 Chalamaiah M, Yu W and Wu J, Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins, A review, *Food Chem*, 2018, **245**, 205–222.

3 Daliri E B, Oh D H and Lee B H, Bioactive Peptides, *Foods*, 2017, **6**, E32.

4 Tu M, Cheng S, Lu W and Du M, Advancement and prospects of bioinformatics analysis for studying bioactive peptides from food-derived protein, Sequence, structure, and functions, *Trends Analyst Chem*, 2018, **105**, 7–17.

5 Aluko R E, Antihypertensive peptides from food proteins, *Annu Rev Food Sci Technol*, 2015, **6**, 235–262.

6 O'Neill S and O'Driscoll L, Metabolic syndrome, a closer look at the growing epidemic and its associated pathologies, *Obesity Rev*, 2015, **16**, 1–12.

7 Muttenthaler M, King G F, Adams D J and Alewood P F, Trends in peptide drug discovery, *Nat Rev Drug Dis*, 2021, **20**, 309–325.

8 Morrison C, Constrained peptides' time to shine?, *Nat Rev Drug Discov*, 2018, **17**, 531–533.

9 Diao L and Meibohm B, Pharmacokinetics and pharmacokinetic–pharmacodynamic correlations of therapeutic peptides, *Clin Pharmacokinet*, 2013, **52**, 855–868.

10 Ebbensgaard A, Mordhorst H, Overgaard M T, Nielsen C G, Aarestrup F M, *et al.*, Comparative evaluation of the antimicrobial activity of different antimicrobial peptides against a range of pathogenic bacteria, *PLOS One*, 2015, **10**, e0144611.

11 Luo X, Chen H, Song Y, Qin Z, Xu L, *et al.*, Advancements, challenges and future perspectives on peptide-based drugs, Focus on antimicrobial peptides, *European J Pharm Sci*, 2023, **181**, 106363.

12 Wang Y, Zhao T, Wei D, Strandberg E, Ulrich A S, *et al.*, How reliable are molecular dynamics simulations of membrane active antimicrobial peptides?, *Biochimica et Biophysica Acta (BBA) – Biomembranes*, 2014, **1838**, 2280–2288.

13 Lee E Y, Lee M W, Fulan B M, Ferguson A L and Wong G C L, What can machine learning do for antimicrobial peptides, and what can antimicrobial peptides do for machine learning?, *Interface Focus*, 2017, **7**, 20160153.

14 Capecchi A, Cai X, Personne H, Köhler T, van Delden C, *et al.*, Machine learning designs non-hemolytic antimicrobial peptides, *Chem Sci*, 2021, **12**, 9221-9232.

15 Khabbaz H, Karimi-Jafari M H, Saboury A A and Ali B B, Prediction of antimicrobial peptides toxicity based on their physico-chemical properties using machine learning techniques, *BMC Bioinformatics*, 2021, **22**, doi: 10.1186/s12859-021-04468-y.

16 Xu J, Li F, Leier A, Xiang D, Shen H H, *et al.*, Comprehensive assessment of machine learning-based methods for predicting antimicrobial peptides, *Briefings Bioinformatics*, 2021, **22**, doi: 10.1093/bib/bbab083.

17 FitzGerald R J, Cermeño M, Khalesi M, Kleekayai T and Amigo-Benavent M, Application of *in silico* approaches for the generation of milk protein-derived bioactive peptides, *J Funct Foods*, 2020, **64**, 103636.

18 Keska P and Stadnik J, Antimicrobial peptides of meat origin - An *in silico* and *in vitro* analysis, *Protein Pept Lett*, 2017, **24**, 165–173.

19 Kartal C, Türköz B K and Otles S, Prediction, identification and evaluation of bioactive peptides from tomato seed proteins using *in silico* approach, *J Food Meas Charact*, 2020, **14**, 1865–1883.

20 Panjaitan F C, Gomez H L and Chang Y W, *In silico* analysis of bioactive peptides released from giant grouper (*Epinephelus lanceolatus*) roe proteins identified by proteomics approach, *Molecules*, 2018, **23**, 2910.

21 Pooja K M, Rani S & Prakash B, *In silico* approaches towards the exploration of rice bran proteins-derived angiotensin-I-converting enzyme inhibitory peptides, *Int J Food Prop*, 2017, **20**(2), 2178-2191.

22 Zhao W, Xue S, Yu Z, Ding L, Li J, *et al.*, Novel ACE inhibitors derived from soybean proteins using *in silico* and *in vitro* studies, *J Food Biochem*, 2019, **43**, e12975.

23 Kajla P, Sharma A and Sood D R, Flaxseed-a potential functional food source, *J Food Sci Technol*, 2015, **52**, 1857–1871.

24 Minkiewicz P, Iwaniak A and Darewicz M, BIOPEP-UWM database of bioactive peptides, current opportunities, *Int J Mol Sci*, 2019, **20**, 5978.

25 Vlieghe P, Lisowski V, Martinez J and Khrestchatisky M, Synthetic therapeutic peptides, science and market, *Drug Discov Today*, 2010, **15**, 40–56.

26 Thundimadathil J, Cancer treatment using peptides, current therapies and future prospects, *J Amino Acids*, 2012, **2012**, 967347.

27 Shaji J and Patole V, Protein and peptide drug delivery, oral approaches, *Indian J Pharm Sci*, 2008, **70**, 269–277.

28 Wang B, Xie N and Li B, Influence of peptide characteristics on their stability, intestinal transport, and *in vitro* bioavailability, A review, *J Food Biochem*, 2018, **43**(1), e12571.

29 Joseph S, Karnik S, Nilawe P, Valadi J and Idicula-Thomas S, ClassAMP, A prediction tool for classification of antimicrobial peptides, *IEEE/ACM Transact Computat Biol Ioinformatics/IEEE, ACM*, 2012, doi: org/10.1109/TCBB.2012.89.

30 Huang S, Cai N, Pacheco P P, Narrandes S, Wang Y, *et al.*, Applications of Support Vector Machine (SVM) learning in cancer genomics, *Cancer Genomics Proteomics*, 2018, **15**(1), 41-51, doi: 10.21873/cgp.20063.

31 Meher P K, Sahu T K, Saini V and Rao A R, Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into Chou's general PseAAC, *Sci Rep*, 2017, **7**, 42362.

32 Schaduangrat N, Nantasenamat C, Prachayasittikul V and Shoombuatong W, Meta-iAVP, A sequence-based meta-predictor for improving the prediction of antiviral peptides using effective feature representation, *Int J Mol Sci*, 2019, **20**, 5743.

33 Agrawal P, Bhalla S, Chaudhary K, Kumar R, Sharma M, *et al.*, *In silico* approach for prediction of antifungal peptides, *Front Microbiol*, 2018, **9**, 323.

34 Høiby N, Ciofu O and Bjarnsholt T, *Pseudomonas aeruginosa* biofilms in cystic fibrosis, *Future Microbiol*, 2010, **5**, 1663–1674.

35 Yadav M K, Jae-Jun S, Singh B P and Vidal J E, Microbial biofilms and human disease, A concise review, in *New and future developments in microbial biotechnology and bioengineering, Microbial Biofilms*, (Elsevier), 2020, 1-13

36 Hancock R E and Sahl H G, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, *Nat Biotechnol,* 2006, **24**, 1551–1557.

37 Sharma A, Gupta P, Kumar R and Bhardwaj A, dPABBs, A novel *in silico* approach for predicting and designing anti-biofilm peptides, *Sci Rep,* 2016, **6**, 21839.

38 Darwish S, Noreddin A, Tiwari R and Elkhatib W F, *In silico* design, synthesis, and *In vitro* evaluation of novel amphipathic short linear peptides against clinically relevant

bacterial biofilms, *Int J Pept Res Ther*, 2019, **25**, 1075–1085.

39 Teng T, Liu J and Wei H, Anti-mycobacterial peptides, from human to phage, *Cell Physiol Biochem*, 2015, **35**, 452–466.

40 Khatun S, Hasan M, Kurata H, Khatun S, Hasan M, *et al.*, Efficient computational model for identification of antitubercular peptides by integrating amino acid patterns and properties, *FEBS Lett*, 2019, **593**, 3029–3039.

41 Agrawal P, Bhagat D, Mahalwal M, Sharma N and| Raghava G P, AntiCP 2.0, an updated model for predicting anticancer peptides, *Brief Bioinformatics*, 2021, **22**(3), bbaa153, doi: org/10.1093/bib/bbaa153.

42 Fatemi M, Ghandehari F, Bahrami S and Tajedin N, *In silico* and *in vitro* studies of cytotoxic activity of different peptides derived from human lactoferrin protein, *J Kermanshah Univ Med Sci*, 2018, **22**, e69544.

43 Shoombuatong W, Schaduangrat N and Nantasenamat C, Unravelling the bioactivity of anticancer peptides as deduced from machine learning, *Exp Clin Sci J*, 2018, **17**, 734–752.

44 Dai Y, Cai X, Shi W, Bi X, Su X, *et al.*, Pro-apoptotic cationic host defense peptides rich in lysine or arginine to reverse drug resistance by disrupting tumor cell membrane, *Amino Acids*, 2017, **49**, 1601–1610.

45 Yamaguchi Y, Yamamoto K, Sato Y, Inoue S, Morinaga T, *et al.*, Combination of aspartic acid and glutamic acid inhibits tumor cell proliferation, *Biomed Res,* 2016, **37**, 153–159.

46 Ramaprasad A S E, Singh S, Gajendra P S R and Venkatesan S, AntiAngioPred, A server for prediction of anti-angiogenic peptides, *Plos One*, 2015, **10**(9), e0136990, doi: org/10.1371/journal.pone.0136990.

47 Thundimadathil J, Cancer treatment using peptides, current therapies and future prospects, *J Amino Acids*, 2012, **2012**, 967347, doi: 10.1155/2012/967347.

48 Gupta S, Ansari H R, Gautam A and Raghava G P, Open source drug discovery consortium, identification of B-cell epitopes in an antigen for inducing specific class of antibodies, *Biol Direct,* 2013, **8**, 27.

49 Dhanda S K, Gupta S, Vir P, Raghava G P S, Prediction of IL4 inducing peptides, *J Immunol R*es, 2013, **2013**, 263952, doi: org/10.1155/2013/263952.

50 Dhanda S K, Vir P and Raghava G P, Designing of interferon-gamma inducing MHC class-II binders, *Biol Direct,* 2013, **8**, 30, doi: org/10.1186/1745-6150-8-30.

51 Hedner T, Sun X, Junggren I L, Pettersson A and Edvinsson L, Peptides as targets for antihypertensive drug development, *J Hypertens Suppl*, 1992, **10**(7), S121-32.